SYMPOSIUM ON INITIATION OF BACTERIAL GROWTH[1]

CONTENTS

I. Introduction

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All of us have assessed bacterial growth in various ways; in fact, our entire discipline encompasses growth in its many manifestations. Yet, if we would understand growth we cannot be indifferent to its main patterns and to the hypotheses we seem to be following as our respective studies have been completed. The central themes which seem to be governing our thoughts relative to microbial growth may need examination again, if not renovation, lest we become so absorbed in detailed studies, which have a limited scope, that we do not look at the broader areas within which we work.

The genesis of chemical compounds in their particular pattern or patterns of synthesis, however interdependent, still forms the basis for much of our thinking. Cell morphology, as well as the spatial organization within the cell, continues to challenge our technical skill and our incisive reasoning as we seek to understand natural laws relating to all aspects of growth.

The manifestation of growth may be seemingly well known but the delineation of its magnitude and its variation, as well as the physicochemical principles which somehow underlie it, may not be so apparent. In these areas of research one may frequently be impressed by the cyclicity with which scientific facts relative to a phase of growth are presumably "new," only to discover that earlier literature had included the information. There is always merit in periodically viewing the broader aspects of growth in which our information falls.

An example from an earlier report (1) (figure 1) will show what is meant. When *Escherichia coli* was inoculated into sterile liquid whole eggs, sharp-frozen at −25 C followed by holding at −9 C, the pattern of growth initiation once the culture was defrosted in a water bath (37 C) was changed. The more rapid growth of the defrosted population, in contrast to the growth initiation of the control, is the observation upon which we might concentrate our attention. Tanguay (2) recently reported a similar phenomenon in a culture of the assay bacterium, *Streptococcus faecalis* R, strain ATCC 8043, which had been defrosted after storage at −40 C for 12 months.

When our culture was plated on the selective medium, MacConkey agar, the pattern of growth initiation was similar to that obtained in a highly fortified medium—yeast-extract, veal-infusion agar (YEVI-agar)—even though the numbers of viable cells had been reduced during exposure at the subfreezing temperature. It is well to reflect that bacteriological methods suitable for the medical field may not be appropriate for the food bacteriologist (3–6).

Several questions can be asked at this point to try to explain the more rapid growth rate of the defrosted cells. Is this a pattern of behavior peculiar to *E. coli* in liquid whole eggs? A negative answer can be given since *Salmonella typhosa*, *Salmonella oranienburg*, and *Staphylococcus aureus* grew faster when defrosted. Since there was a general pattern, what concepts of growth appear pertinent?

A microbial geneticist has said that "selection of the fast growers" has occurred but what does he really mean? Such an expression implies that prior to the physical insult of a subfreezing temperature there must have been "slow" and "fast" growers in the same pure culture yet it is

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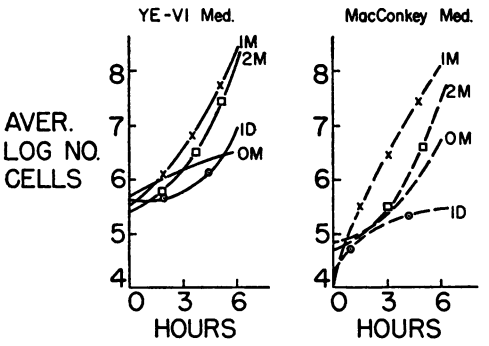

Figure 1. Evidence of the stimulation of *Escherichia coli* by storage at −9 C. *M* signifies months of storage; *1 D*, one day of storage; and *hours*, the period after thawing.

TABLE 1

Generation time for Escherichia coli no. 21

Days*	*g* Time
	min.
0	36.4
7	29.4
14	30.3
21	*22.4*
28	23.6
35	28.4
42	27.6
49	33.0

* At subfreezing temperature.

not clear what type of genotypic expression exists for these respective growth characteristics. "Slow" or "fast" designations (*g* times) for *different* pure cultures have been made (7–10), but are there different *g* times for different cells in the same culture? The data of Powell (11) suggest that there are, yet there is need for more definite information on this point. One might expect that the so-called "slow growers" would be the cells which had persisted because of adjustment to the subfreezing environment. Our evidence also suggests that the "fast growers" would have to have been endowed simultaneously with a short generation time *and* an increased resistance at subfreezing temperatures. Is this logical? Cyclic applications of differing temperatures to cultures offer interesting concepts relative to a rapid rise in the curve of growth initiation. If we assume that the growth pattern of the population is controlled, chiefly, by the generation time, then, theoretically, purposeful alteration of *g* would be the next step.

This approach was tried with *E. coli* exposed to subfreezing temperature in egg mélange and the following generation times were obtained (table 1). At each sampling time, a sample was removed, defrosted, and incubated at 37 C in a water bath. Samples were removed at 0, 3, and 6 hr and plated on YEVI-agar. The *g* time was determined when the population was in the logarithmic phase.

One is tempted to conclude that after 21 days *g* had been shortened. We then asked—would this change persist? Continued insult at the subfreezing temperatures "returned" (?) the generation time to what it had been initially. Selection? One cannot be so sure.

Then followed another approach toward understanding the phenomenon. Attempts to select *E. coli* colonial types, *e.g.*, *GG* for "ground glass," *FI* for "flat irregular," from the same pure culture resulted in substrains with different respective *g* times—presumably the "slow" and the "fast" growers. These have not yet been subjected to the above type of experimentation, although mixtures of the respective colonial types, when frozen and stored, showed an ascendency of one type over another type as growth was initiated. This result could not be correlated with the idea of selection of the "fast growers."

Bacterial physiologists offer the possible explanation that *E. coli* in the original experiment initiated growth faster because the temperature limited a certain enzyme which was essential in the sequential nature of the synthesis of protoplasm before division could occur. The influence of the Ledingham (12) and Penfold (13) theories of lag is at once seen. The step-wise process of synthesis, with its gradients, its enzyme velocities, its polycondensations which must occur, and the microclimate about the cell as well as other aspects of the cellular environment, offer interesting prospects for meaningful information. The *sequential* nature of individual or interdependent reactions is our traditional approach to the study of such patterns yet the *simultaneous* reactions which occur as the dynamic state changes within the cell constitute the area wherein new methods are sought and frontier thinking should become more incisive.

Looking at the original data in another way,

one might say that the rapid rise in the growth rate is but another expression of Weigert's law (14). In 1896, he first observed that when certain tissues regenerate they *respond by producing more than would be required for the reestablishment of the status quo ante.* He expressed the hope that we might one day have the methods to explain why these phenomena appear. It is challenging to consider that physical insult, no matter what the type, might elicit some common patterns of behavior when growth initiation occurs. Should we also consider that regeneration processes as they occur in plants and in animals are basically similar in bacteria? A so-called natural law would seem to be involved if comparative biology, especially comparative biochemistry, could elucidate it. In preparation for regeneration following sublethal treatment, viable cells in the inoculum should accumulate enzymes, and should have available essential metabolites and coenzymes. Perhaps the cell should even leak a few substances before or as the growth pattern takes form and the cell should have a physical state conducive to dynamic events—*all* of these relationships transpiring before synthesis could be accelerated.

Intriguing also would be the thought that storage at subfreezing temperatures creates a special type of dormant state in a nonsporulating species. A synthesis, even at subfreezing temperatures, might allow substance *A* to be utilized in product *B* and this product utilized in product *C* but the use of product *C* to form *D*, which latter product is essential to cellular division, could not occur until the cells were defrosted. If this conception of events is true, product *C* should accumulate, as the cells are held in storage, and could be isolated if appropriate techniques were available. Recent data suggest that this concept is tenable.

Changes in cellular architecture were not noted in the *E. coli* experiments, perhaps because techniques were not available to indicate the subtle changes. That the cells may be altered tinctorially following physical insult is sometimes difficult to observe in a gram-negative species. One may be tempted to demonstrate, through the use of specialized procedures, the disappearance of granules in the physically insulted cells as well as the creation of an altered physical state. *E. coli*, when frozen and thawed, can be shown to be somewhat more susceptible to the lytic action of lysozyme, acting alone or concomitantly with trypsin (15, 16); thus, permeability characterization of physically insulted cells would seem to be much needed. When such treatment has been applied to cells we reflect on the possibility—does resistance always require a new type of cellular organization if the species is to persist?

Our thought for this symposium was that we might point out some of the meaningful studies of the past and, through an exchange of views, evolve other concepts and potentially productive areas for future study.

PROSPECTUS

Our effort in this symposium has been to concentrate on some of the examples of early bacterial growth. By so doing, the participants with their informed thinking and incisive reasoning have also indicated some, though not all, of the research strategy which may be productive in the future. No doubt, we shall continue to search for the "essential" reaction, the "major" components, and "required" metabolite, the "endogenous pools," the spatial and geometric patterns which seem to govern the dynamic behavior of bacterial cells (individually, in populations, and as associates) so that we might improve our understanding and exploitation of natural laws.

Among the more interesting challenges to bacteriologists are the causes of cell division, the factors that may change the generation time of a cell, the phenotypic expression of lag both biochemically and in terms of the architecture of the cell, the assessment of enzyme decay in the early aging of the cell, the disappearance of various metabolic pools as well as the molecular biology which led to their establishment in the beginning, and the creation of the dormant state and its persistence. Of interest also will be the morphological expressions within the cell that reflect the ebb and flow of reaction patterns, the processes that permit the group-translocation of molecules, the carrier or transfer systems, and the multiple interdependent reactions which lead to reactivation after physical insult or after chemical inhibition, so that there is development and persistence of a cell, alone or in a group in its microclimate. The processes which create the imbalance, wherein regeneration occurs but aging and death must result, likewise

might have sequences which, if studied thoroughly, are as individualistic as synthesis.

In the near future we may find more general acceptance of a rearranged terminology to describe what we mean, *e.g.*, replication or storage compartment, as we elucidate natural differences and similarities among species.

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